

<p align="center"><b>APPENDIX B: REAGENTS</b></p>	<p align="center">Page 1 of 18</p>
<p align="center"><b>FLUORESCENT DETECTION PCR-BASED STR DNA PROTOCOL:POWERPLEX® 16 BIO SYSTEM - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION III</b></p>	<p align="center">Issue No. : 3</p>
	<p align="center">Effective Date: 6-March-2006</p>
<p><b>APPENDIX B: REAGENTS</b></p> <p>1. This appendix describes the preparation of reagents necessary for the DNA analysis. For each reagent listed, the company and catalog number is included. As a reagent is prepared, it will be labeled to include the following information:</p> <p style="padding-left: 40px;">Identity Concentration Lot number Date of preparation Initials of preparer Date of expiration</p> <p>and if appropriate:</p> <p style="padding-left: 40px;">Date of autoclaving Storage requirements</p> <p>2. All reagents will be prepared with Type I water, unless other wise stated.</p> <p>3. All chemicals and reagents will be stored according to the manufacturers' specifications. All chemicals containing biologicals will be disposed of in biohazard bags. Waste organic reagents and Ethidium Bromide will be placed in designated waste containers in a hood and removed from the laboratory by an appropriate means of disposal. All other reagents can be disposed of in the laboratory sink.</p> <p>4. Any changes in chemical supply companies will be carefully checked by the Section Supervisor to ensure the chemical being provided meets the specifications necessary for the reagent. Any changes in chemical supply companies will be brought to the Forensic Biology Section Chief's attention so the list can be updated as necessary.</p> <p>5. Concentrations preceding reagent components reflect the final concentrations of that specific component in the resulting mixture.</p> <p>6. When a reagent is diluted and used by a number of examiners and retained, a lot number must be created and the reagent must be traceable to the reagent log. The container with the diluted reagent must contain the reagent stock lot number, the date the dilution was prepared, and the initials of the individual preparing the dilution. If a stock solution is diluted and used once and discarded or the dilution is used only by the examiner preparing the dilution, a new lot number does not need to be assigned or recorded in the reagent log. The Gold ST®R 10X Buffer and the Control DNA (9947A) are examples of such reagents where this applies. The original reagent stock lot number will be used.</p>	

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	Effective Date: 6-March-2006
ACETIC ACID - GLACIAL	
Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date	
Purchased from Fisher Scientific, Fair Lawn, NJ., Catalog number A507-212.	
ACETIC ACID, 0.5% IN 95% ETHANOL	
Expiration date: Six months from date of preparation	
	100 mL                      200 mL
Glacial acetic acid	500 µL                      1 mL
95% ethanol	99.5 mL                      199 mL
Add the glacial acetic acid to 95% ethanol and mix well.	
AGAROSE - DNA TYPING GRADE	
Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date	
Purchased from Invitrogen, Carlsbad, CA, Catalog number 14610-018, 500 g bottle.	
Also purchased from Bio-Rad, Melville, NY., Catalog number 162-034, 500 g bottle.	
AGAROSE - 3:1 NUSIEVE	
Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date	
Purchased from BMA, Rockland, ME., Catalog number 50090, 125 g bottle.	
ALUQUANT™ HUMAN DNA QUANTITATION KIT	
Expiration date: Manufacturer's expiration date.	
Purchased from Promega Corporation, Madison, WI., Catalog Number DC1011 for 400 reaction kit.	
Store all AluQuant™ Human Quantitation System reagents at -20°C.	
Kit components included (expiration date same as kit unless specified):	
<ul style="list-style-type: none"><li>• ALUQUANT™ ENZYME SOLUTION</li><li>• ALUQUANT™ DENATURATION SOLUTION</li><li>• ALUQUANT™ NEUTRALIZATION SOLUTION</li><li>• ALUQUANT™ PROBE MIX</li><li>• ENLITEN® LUCIFERASE/LUCIFERIN (L/L) REAGENT</li></ul>	
Expiration date: AluQuant™ Human Quantitation System kit expiration date when in lyophilized state. Once reconstituted discard after two weeks.	

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<p>Equilibrate to room temperature prior to use. Add the ENLITEN® Luciferase/Luciferin (L/L) Reconstitution Buffer to the ENLITEN® Luciferase/Luciferin Reagent in the brown bottle and then invert the bottle several times to mix.</p> <p>Store unused portions of Enliten® Luciferase/Luciferin (L/L) Reagent in a -20°C up to two weeks.</p> <ul style="list-style-type: none"> <li>• ENLITEN® LUCIFERASE/LUCIFERIN (L/L) RECONSTITUTION BUFFER - Equilibrate to room temperature prior to use.</li> <li>• HUMAN GENOMIC DNA STANDARD (20 ng/μL) -Prepare dilution of the Human Genomic DNA Standard as follows:</li> </ul> <p>NOTE: Dilution series should be made up fresh each use.</p> <ol style="list-style-type: none"> <li>1. Label seven 0.5 mL autoclaved tubes (4 ng/μL, 2 ng/μL, 1 ng/μL, 0.5 ng/μL, 0.25 ng/μL, 0.125 ng/μL, and 0.062 ng/μL)</li> <li>2. Vortex the Human Genomic DNA Standard (20 ng/μL) to mix it thoroughly.</li> <li>3. Pipette into the tube labeled 4 ng/μL (tube 1) 60 μL of TE<sup>-4</sup> Buffer and 15 μL of Human Genomic DNA Standard; vortex well.</li> <li>4. Pipette into the tube labeled 2 ng/μL (tube 2) 40 μL of TE<sup>-4</sup> Buffer and 40 μL of sample from tube 1. Vortex well</li> <li>5. Pipette into the tube labeled 1 ng/μL (tube 3) 40 μL of TE<sup>-4</sup> Buffer and 40 μL of sample from tube 2. Vortex well</li> <li>6. Pipette into the tube labeled 0.5 ng/μL (tube 4) 40 μL of TE<sup>-4</sup> Buffer and 40 μL of sample from tube 3. Vortex well</li> <li>7. Pipette into the tube labeled 0.25 ng/μL (tube 5) 40 μL of TE<sup>-4</sup> Buffer and 40 μL of sample from tube 4. Vortex well</li> <li>8. Pipette into the tube labeled 0.125 ng/μL (tube 6) 40 μL of TE<sup>-4</sup> Buffer and 40 μL of sample from tube 5. Vortex well</li> <li>9. Pipette into the tube labeled 0.062 ng/μL (tube 7) 40 μL of TE<sup>-4</sup> Buffer and 40 μL of sample from tube 6. Vortex well</li> <li>10. Pipette in the tube labeled control (tube 8) 40 μL of TE<sup>-4</sup> Buffer.</li> </ol> <ul style="list-style-type: none"> <li>• HYDROCHLORIC ACID (250 mM)</li> </ul> <p>Prepare a 200 mM HCl solution by briefly vortexing the 250 mM HCl solution and then placing 12 mL of the solution into a 15 mL conical tube. Subsequently add 3 mL of sterile Type I water and mix by inverting several times. The HCl solution can be stored at room temperature.</p> <ul style="list-style-type: none"> <li>• NUCLEASE-FREE WATER</li> </ul>	

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AMMONIUM PERSULFATE	
Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date	
NOTE: Prepare on the day of use	
Based on the volume needed, add 0.1g increments of ammonium persulfate to a 15 mL conical vial. Add 1 mL Type I Water per 0.1 g of ammonium persulfate.	
Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number A6761, 250 g bottle.	
AMPLITAQ™ GOLD DNA POLYMERASE	
Expiration date: Manufacturer's expiration date	
Purchased from Applied BioSystems, Foster City, CA., Catalog number N808-0243 (6 pack) or N808-0244 (12 pack).	
BIND SILANE	
Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date	
Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number M-6514, 25 mL bottle.	
CALCUM CHLORIDE, 1M	
Expiration date: Six months from the date of preparation	
MW	100 mL
CaCl <sub>2</sub>	110.9
Type I Water	11.09 g
	90 mL
Dissolve the appropriate amount of CaCl <sub>2</sub> into Type I Water. Bring up to final volume	
CaCl <sub>2</sub> - Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number C1016, 100 g bottle.	
CALCUM CHLORIDE BUFFER, 1X	
Expiration date: Six months from date of preparation	
MW	100 mL
1M Tris	121.1
1 M CaCl <sub>2</sub>	5 mL
Type I water	110.9
	1 mL
	94 mL
Mix well. May be stored at room temperature.	

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DITHIOTHREITOL, 0.39 M (DTT)	
Expiration date: Six months from date of preparation	
MW 25 mL	
DTT 154.2 1.50 g	
Type I Water 15 mL	
Add DTT to Sterile Type I Water and mix well. When DTT is completely dissolved, bring up to final volume with Sterile Type I Water. Filter sterilize and divide into 500 µL aliquots in sterile microcentrifuge tubes and store at -20 C.	
DTT (Molecular Biology Grade) - Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number D0632, 25 g bottle.	
DNA IQ™ SYSTEM	
Expiration date: Manufacturer's expiration date	
Purchased from Promega Corporation, Madison, WI., Catalog Number DC6700 for 400 reaction kit.	
Store all DNA IQ™ Isolation System reagents at room temperature.	
Kit components included (expiration date same as kit unless specified):	
• DNA IQ™ LYSIS BUFFER	
On the day of use add 2.5 µL of 0.39 M DTT for every 100 µL of DNA IQ™ Lysis Buffer that is prepared.	
• DNA IQ™ RESIN	
• DNA IQ™ WASH BUFFER	
Add 35 mL of 95% ethanol and 35 mL of Isopropyl Alcohol to 2X Wash Buffer. Be certain to accurately measure the alcohol volumes since this could negatively impact the performance of the extraction. Mix contents and store at room temperature in a tightly capped container.	
• DNA IQ™ ELUTION BUFFER	
ETHANOL, 95%	
Expiration date: Dispose of when necessary	
Purchased as Reagent Alcohol (190 Proof) from VWR, Bridgeport, NJ, Catalog number 6590-1, 4 L bottle.	

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**ETHIDIUM BROMIDE (EtBr), 5 mg/mL****WARNING!**

ETHIDIUM BROMIDE IS MUTAGENIC. ALWAYS WEAR GLOVES WHEN HANDLING EtBr.

Expiration date: Six months from the date of preparation

	25 mL	50 mL	100 mL
Ethidium Bromide	0.125 g	0.25 g	0.5 g
Type I Water	20 mL	40 mL	80 mL

Add EtBr to Type I Water. When EtBr is in solution, bring up to final volume. (Caution: EtBr is light sensitive. Be careful not to expose it to light for any extended period of time.) Store in a brown bottle or foil wrapped container at room temperature.

Ethidium Bromide - Purchased from VWR, Bridgeport, NJ., Catalog number 7738, 100 g bottle.

Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number E8751, 5 g bottle.

NOTE: Ethidium Bromide may also be made using ethidium bromide solution (10 mg/mL) in a 1:2 dilution with Type I Water (10 mL EtBr + 10 mL Type I Water = 20 mL EtBr solution, 5 mg/mL).

Ethidium Bromide Solution (10 mg/mL) - purchased from GIBCO BRL, Grand Island, NY Catalog number 5585UA, 10 mL bottle.

**ETHYLENEDIAMINETETRAACETIC ACID (EDTA), 0.5 M**

Expiration date: Six months from date of preparation

	MW	500 mL	1L	2 L
EDTA	372.2	93.05 g	186.1 g	372.2 g
Type I Water		375 mL	750 mL	1.5 L

Add EDTA to Type I Water. Mix well and pH to 8.0 with 10 N NaOH (EDTA will not go into solution unless pH = 8.0). When totally dissolved, bring up to final volume with Type I Water and recheck pH. Dispense into appropriate container and autoclave at 215 F at 20 lb for 20 minutes.

EDTA (Molecular Biology Grade) - Purchased from Sigma Chemical Co., St. Louis, MO., Catalog number E 5134, 500 g bottle.

EDTA (Disodium Salt) purchased from Invitrogen, Carlsbad, CA , Catalog number 15575-028, 500 g bottle.

**GENE PAGE PLUS™ GEL SOLUTION, 6.0%**

Expiration date: Manufacturer's expiration date

Purchased from Amresco Branded Product Group, Solon, OH., Catalog number E568, 500 mL bottle (800-892-2805).

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GLYCEROL

Expiration date: Manufacturer’s expiration date if listed, otherwise no expiration date

Purchased from Invitrogen, Carlsbad, CA. Catalog Number 15514-011, 500 mL bottle.

8-HYDROXYQUINOLINE

Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date

Purchased from VWR, Bridgeport, NJ., Catalog number 4969-125\*NY, 125 gram bottle.

LADDER, 123 BASE PAIR

Expiration date: Twelve months from the date of receipt when stored at -20<sup>0</sup>C

Purchased from Invitrogen, Carlsbad, CA, Catalog number 15613-011, 100 ug vial.

LOADING BUFFER, 5X

Expiration date: No expiration date. Discard as needed.

Stock	20 mL	50 mL
Bromophenol Blue	0.02 g	0.05 g
Glycerol	10 mL	25 mL
0.5 M EDTA	4 mL	10 mL
Sterile 1X TE-4 Buffer	6 mL	15 mL

Combine components and mix well. Divide into 1 mL aliquots and store at -20 C. One aliquot may be left at room temperature for daily use.

Bromophenol Blue - Purchased from Sigma Chemical Co., St. Louis, MO, Catalog Number B5525, 10 g bottle.

For Glycerol, EDTA and TE-4 Buffer see this list.

PAGE PLUS™ GEL SOLUTION, 40%

Expiration date: Manufacturer's expiration date

Purchased from Amresco Branded Product Group, Solon, OH., Catalog number E562, 100 or 500 mL bottle (800-892-2805).

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<p>PCR DIGESTION BUFFER, 1.0%</p> <p>Expiration date: Six months from date of preparation</p> <table border="0"> <tr> <td>Stock</td><td>100 mL</td></tr> <tr> <td>1 M Tris</td><td>1 mL</td></tr> <tr> <td>0.5 M EDTA</td><td>2 mL</td></tr> <tr> <td>NaCl</td><td>0.29 g</td></tr> <tr> <td>20% SDS</td><td>5 mL</td></tr> <tr> <td>Type I Water</td><td>91mL</td></tr> </table> <p>Mix all reagents together and pH to 7.5 using dilute HCL. Adjust to final volume with Type I Water and store at room temperature.</p> <p>PHENOL-CHLOROFORM-ISOAMYL ALCOHOL, 25:24:1</p> <p>Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date</p> <p>To aid visualization add approximately 0.07125 g of 8-Hydroxyquinoline/100 mL of phenol-chloroform-isoamyl alcohol solution until the phenol turns slightly yellow in color. CAUTION: Measure 8-Hydroxyquinoline under hood. Store at 2-5 C.</p> <p>Purchased from Sigma Chemical Co., St. Louis, MO Catalog number P 3803, 100 and 400 mL bottle.</p> <p>Purchased from Invitrogen, Carlsbad, CA, Catalog number 5593UB, 400 mL bottle.</p> <p>PHOSPHATE-BUFFERED SALINE (PBS), 1X, pH 7.2</p> <p>Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date</p> <p>Purchased from Cambrex, Walkersville, MD., Catalog # 17-516Q 1L bottle.</p> <p>POWERPLEX® 16 BIO SYSTEM KIT</p> <p>Expiration date: Manufacturer's expiration date</p> <p>Purchased from Promega, Madison, WI., Catalog Number DC6541 for 100 reaction kit or DC6540 for 400 reaction kit.</p> <p>Kit components included (expiration date same as kit unless specified):</p> <ul style="list-style-type: none"> <li>• POWERPLEX® 16 BIO PRIMER PAIRS</li> <li>• POWERPLEX® 16 BIO ALLELIC LADDER</li> <li>• STR LOADING BUFFER, 2X (TRACKING DYE)</li> <li>• MATRIX 16 BIO</li> <li>• GOLD ST®R 10X BUFFER - Purchased from Promega, Madison, WI. Catalog Number DM2411, 1.2 mL tube</li> <li>• FLUORESCENT INTERNAL LANE STANDARD 600 BIO</li> <li>• CONTROL DNA (GM9947A CELL LINE) - Purchased from Promega Corporation, Madison, WI., 250 ng tube, 10 ng/µL, Catalog number DD1001.</li> <li>• BROMOPHENOL BLUE LOADING BUFFER</li> </ul>		Stock	100 mL	1 M Tris	1 mL	0.5 M EDTA	2 mL	NaCl	0.29 g	20% SDS	5 mL	Type I Water	91mL
Stock	100 mL												
1 M Tris	1 mL												
0.5 M EDTA	2 mL												
NaCl	0.29 g												
20% SDS	5 mL												
Type I Water	91mL												

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PROTEINASE K ENZYME (20 mg/mL)

Expiration date: Six months from date of preparation

Stock	5 mL	15 mL	25 mL
Proteinase K	100 mg	300 mg	500 mg
Sterile Type I Water	5 mL	15 mL	25 mL

Add lyophilized Proteinase K to appropriate amounts of sterile Type I Water. When completely reconstituted, divide into 250 µL aliquots in microcentrifuge tubes and store at -20 C. Thaw at room temperature prior to use and keep on ice once thawed.

Purchased from Invitrogen, Carlsbad, CA., Catalog number 25530-031, 1.0 g bottle. Store dry at 2-5 C.

Purchased from Sigma Chemical CO., St. Louis, MO, Catalog number P 2308, 100 mg bottle.

QIAamp® DNA BLOOD MINI KIT

Expiration date: Refer to individual kit component expiration dates.

Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 51104 for 50 sample kit, 51106 for 250 sample kit, or 51108 for 1000 sample kit.

Kit components included:

- QIAGEN® AE ELUTION BUFFER - Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 19077.

Expiration date: Twelve months from date of receipt into the laboratory

- QIAGEN® AL LYSIS BUFFER - Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 19075.

Expiration date: Twelve months from date of receipt into the laboratory

- QIAGEN® AW1 WASH BUFFER (concentrate) - Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 19081.

Expiration date: Twelve months after dilution with ethanol

Before initial use, dilute concentrate with 95% ethanol according to the following table:

Kit Size	AW1 concentrate	Ethanol	Final volume
50	19 mL	25 mL	44 mL
250	95 mL	125 mL	220 mL
1000	175 mL	230 mL	405 mL

Once the QIAGEN® AW1 wash buffer has been diluted with ethanol, store the buffer at room temperature.

- QIAGEN® AW2 WASH BUFFER (concentrate) - Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 19072.

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	Effective Date: 6-March-2006		
Expiration date: Twelve months after dilution with ethanol			
Before initial use, dilute concentrate with 95% ethanol according to the following table:			
Kit Size	AW2 concentrate	Ethanol	Final volume
50	13 mL	30 mL	43 mL
250	66 mL	160 mL	226 mL
1000	127 mL	300 mL	427 mL
Once the QIAGEN® AW2 wash buffer has been diluted with ethanol, store the buffer at room temperature.			
<ul style="list-style-type: none"><li>QIAGEN® PROTEASE - Purchased from QIAGEN, Inc., Valencia, CA, Catalog number 19155 (125 mg) or 19157 (4 x 125 mg).</li></ul>			
Expiration date: 6 months after sample is reconstituted if stored at –20°C. 2 months after sample is reconstituted if stored at 4°C.			
Reconstitute with supplied QIAGEN® protease solvent before initial use according to the table below:			
Kit Size	QIAGEN® Protease	Protease solvent	
50	24 mg	1.2 mL	
250	110 mg	5.5 mL	
1000	4 x 110 mg	4 x 5.5 mL	
<ul style="list-style-type: none"><li>QIAGEN® PROTEASE SOLVENT</li></ul>			
Expiration date: Twelve months from date of receipt into the laboratory			
QIAamp® 96 DNA BLOOD KIT			
Expiration date: Refer to individual kit component expiration dates as specified under QIAamp® DNA BLOOD MINI KIT			
Purchased from QIAGEN, Inc., Valencia, CA, Catalog # 51161 for 4 plate kit, or #51162 for 12 plate kit.			
Kit components included:			
<ul style="list-style-type: none"><li>QIAGEN® AE ELUTION BUFFER</li><li>QIAGEN® AL LYSIS BUFFER</li><li>QIAGEN® AW1 WASH BUFFER (concentrate)</li><li>QIAGEN® AW2 WASH BUFFER (concentrate)</li><li>QIAGEN® PROTEASE</li><li>QIAGEN® PROTEASE SOLVENT</li></ul>			

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QUANTITATION STANDARDS - K562 DNA, UNCUT	
Expiration date: Manufacturer's expiration date	
Purchased from Invitrogen, Carlsbad, CA., Catalog number 4420SA, 300 µL each. Standards include 500 ng, 250 ng, 125 ng, 63 ng, 31 ng and 15 ng.	
SARKOSYL, 20%	
Expiration date: Six months from date of preparation	
	MW250 mL500 mL2 L
N-Lauroylsarcisine	293.450 g100 g400 g
Type I Water	200 mL400 mL1600 mL
Add the appropriate amount of N-Lauroylsarcisine to Type I Water and mix until completely dissolved and the solution is clear. Bring up to volume with Type I Water, filter sterilize and store in sterile bottles at room temperature.	
N-Lauroylsarcisine, Sodium Salt - Purchased from Sigma Chemical Co., St, Louis, MO., Catalog number L 5125, 500 g bottle.	
SDS (SODIUM DODECYL SULFATE), 0.1%	
Expiration date: Six months from date of preparation	
Add 5 mL of 20% SDS solution to 995 mL of Type 1 Water.	
SDS (SODIUM DODECYL SULFATE), 20%	
Expiration date: Six months from date of preparation	
	MW1 L2 L
20% SDS	288.3200 g400 g
Type I Water	750 mL1500 mL
CAUTION: AN AEROSOL MASK OR FUME HOOD SHOULD BE USED WHEN MAKING THIS SOLUTION.	
Add the appropriate amount of SDS to Type I Water and mix until SDS is completely into solution and solution is clear, not cloudy. Adjust to final volume with Type I Water and store at room temperature.	
SDS - Purchased from VWR, Bridgeport, NJ., Catalog number 7718, 100 g bottle.	

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SODIUM CHLORIDE (NaCl), 5 M					
Expiration date: Dispose of when necessary					
	MW	500 mL	1 L	4 L	16 L
NaCl	58.44	146.1 g	292.2 g	1168.8 g	4675.2 g
Type I Water		500 mL	1 L	4 L	16 L
Begin with 60-75% of the total volume of Type I Water in a beaker on a stir plate and add the NaCl slowly. Next add almost all of the required volume of Type I Water because it will be needed in order for the NaCl to go into solution. When the NaCl is dissolved, bring up to final volume by adding Type I Water. Larger volumes (4 L) that are to be used for denaturation solutions need not be autoclaved. NaCl for all other uses must be dispensed into appropriate containers and autoclaved at 215 F for 20 minutes at 20 lb.					
NaCl - Purchased from VWR, Bridgeport, NJ., Catalog number 7532, 12 kg box.					
NaCl, 5 M - Purchased from Invitrogen, Carlsbad, CA., Catalog number 4740UB, 10 liter bottle.					
SODIUM HYDROXIDE (NaOH), 2 N					
Expiration date: Six months from date of preparation.					
	MW	2 L	20 L		
10 N NaOH	40.0	400 mL	4 L		
Type I Water		1600 mL	16 L		
Purchased from VWR, Bridgeport, NJ., Catalog number H385, 4 liter bottle.					
Combine components and mix well. Store at room temperature.					
SODIUM HYDROXIDE (NaOH), 5 N					
Expiration date: Dispose of when necessary					
	MW	1 L			
10 N NaOH	40.0	500 mL			
Type I Water		500 mL			
Begin with 60-75% of the total volume of Type I Water in a beaker on a stir plate and add the NaOH slowly. Bring up to final volume by adding Type I Water.					
Purchased from VWR, Bridgeport, NJ., Catalog number H385, 4 liter bottle.					

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SODIUM PHOSPHATE, MONOBASIC, MONOHYDRATE (NaH2PO4-H2O)			
Expiration date: Manufacturer's expiration date			
Purchased from VWR, Bridgeport, NJ., Catalog number 7892, 500 g bottle.			
SSPE, 25 X			
Expiration date: Six months from date of preparation			
	MW	1 L	2 L
4.5 M NaCl	58.44	262.98 g	438.3 g
250 mM NaH2PO4-H2O	138.0	34.5 g	69.0 g
25 mM EDTA	372.2	9.3 g	18.61 g
Type I Water		450 mL	900 mL
Add the EDTA to Type I Water, and let it dissolve completely. (It is necessary to pH to 8.0 with 10 N NaOH in order for the EDTA to go into solution.) Once dissolved, add the NaH2PO4-H2O first and the NaCl second. Add Type I Water until 95% of the total volume is reached. When all solids are dissolved, pH to 7.4 with NaOH (will take about 20-25 mL NaOH/liter). Bring the solution up to total volume with Type I Water. The SSPE should be dispensed into bottles and autoclaved. Store at room temperature.			
For NaCl, EDTA and NaOH see this list.			
STAIN EXTRACTION BUFFER			
Expiration date: Six months from date of preparation			
	MW	1 L	2 L
Tris	121.1	1.21 g	2.42 g
EDTA	372.2	3.72 g	7.44 g
NaCl	58.44	5.84 g	11.68 g
Type I Water		600 mL	1200 mL
Adjust pH to 8.0 with NaOH			
20% SDS	288.3	100 mL	200 mL
Adjust to final volume with Type I Water and store at room temperature.			
For Tris, NaCl and EDTA see this list.			

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FLUORESCENT DETECTION PCR-BASED STR DNA PROTOCOL:POWERPLEX® 16 BIO SYSTEM - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION III		Issue No. : 3	
		Effective Date: 6-March-2006	
TAE, 1X			
1:19 dilution of 20X TAE			
Expiration date: Six months from date of preparation			
	2 L	4 L	
20X TAE	100 mL	200 mL	
Type I water	1900 mL	3800 mL	
1:50 dilution of 50X TAE			
Expiration date: Six months from date of preparation			
	2 L	20 L	
50X TAE	40 mL	400 mL	
Type I water	1960 mL	19.6 L	
TAE, 20X			
Expiration date: Six months from date of preparation			
	MW	2 L	4 L
Tris	121.1	193.2 g	386.4 g
Glacial Acetic Acid		45.6 mL	91.2 mL
0.5 M EDTA, pH 8.0		80.0 mL	160.0 mL
Type I Water		1874.4 mL	3748.8 mL
Begin with 60-75% of the total volume of Type I Water and add the appropriate amounts of the above chemicals; mix well. When dissolved, pH solution to 7.9 with glacial acetic acid. Bring up to final volume and store in bulk. Autoclave for 20 minutes.			
Glacial Acetic Acid - Purchased from Fisher Scientific, Fair Lawn, NJ Catalog number A-38, 500 mL bottle.			
For EDTA and Tris see this list.			

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FLUORESCENT DETECTION PCR-BASED STR DNA PROTOCOL:POWERPLEX® 16 BIO SYSTEM - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION III		Issue No. : 3	
		Effective Date: 6-March-2006	
TAE, 50X			
Expiration date: Six months from date of preparation.			
Stock	MW	1 L	2 L
Tris	121.1	241.5 g	483 g
Glacial Acetic Acid		57 mL	114 mL
0.5 M EDTA, pH 8.0		100 mL	200 mL
Type I Water		500 mL	1 L
Add components to Type I Water and adjust to pH 7.9 with additional acetic acid. Adjust to final volume with Type I Water. Autoclave at 215 C and 20 lbs pressure for 20 minutes. Store at room temperature.			
Expiration date: Six months for date of receipt			
Purchased from Invitrogen, Carlsbad, CA, Catalog number 4710UA, 1 liter bottle.			
TBE, 0.5X			
1:40 dilution of 20X TBE			
Expiration date: Six months from date preparation			
	2L	20 L	
20X TBE	50 mL	500 mL	
Type I water	1950 mL	19.5 L	
TBE, 1X			
1:10 dilution of 10X TBE			
Expiration date: Six months from date preparation			
	2 L	20 L	
10X TBE	200 mL	2 L	
Type I water	1800 mL	18 L	
1:20 dilution of 20X TBE			
Expiration date: Six months from date preparation			
	2L	4L	
20X TBE	100 mL	200 mL	
Type I water	1900 mL	3800 mL	

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FLUORESCENT DETECTION PCR-BASED STR DNA PROTOCOL:POWERPLEX® 16 BIO SYSTEM - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION III	Issue No. : 3		
	Effective Date: 6-March-2006		
TBE, 10X			
Expiration date: Manufacturer's expiration date			
Purchased from Invitrogen, Carlsbad, CA., Catalog Number 15581-028, 10 liter container/box.			
TBE, 20X			
Expiration date: Six months from date of preparation			
	MW	2 L	
Tris	121.1	432 g	
Boric Acid	61.83	220 g	
0.5 M EDTA	372.2	160 mL	
Type I Water		1000 mL	
Bring up to final volume and store at room temperature.			
For Tris and Na <sub>2</sub> EDTA-2H <sub>2</sub> O see this list.			
Boric Acid - Purchased from Sigma Chemical Co., St. Louis, MO. Catalog number B-0394.			
TEMED			
Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date			
Purchased from Sigma Chemical Co., St. Louis, MO. Catalog number T-8133, 100 mL bottle.			
TNE			
Expiration date: Six months from date of preparation			
	MW	500 mL	1 L
Tris	121.1	0.605 g	1.21 g
NaCl	58.44	2.92 g	5.84 g
EDTA	372.2	0.185 g	0.37 g
Type I Water		450 mL	900 mL
Combine components and mix until completely dissolved. Bring up to final volume with Type I Water and store at room temperature.			
For Tris, NaCl and EDTA see this list.			

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**FLUORESCENT DETECTION PCR-BASED STR  
DNA PROTOCOL: POWERPLEX® 16 BIO SYSTEM - FORENSIC  
BIOLOGY SECTION PROCEDURE MANUAL,  
SECTION III**

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**TRIS, 1 M, pH 7.5**

Expiration date: Six months from the date of preparation

	MW	500 mL	1 L	2 L
Tris 1 M	121.1	60.55 g	121.10 g	242.2 g
Tris 2 M	121.1	121.10 g	242.20 g	484.4 g
Type I Water		300 mL	600 mL	1200 mL

Dissolve the appropriate amount of Tris base into Type I Water. Mix well and adjust to pH 7.5 with 37.1% HCL. Bring up to final volume and recheck pH. Dispense into bottles and autoclave at 215 F for 20 minutes. Store at room temperature.

Tris - Purchased from VWR, Bridgeport, NJ., Catalog number 7732, 2.5 kg box.

For HCl see this list.

**TRIS-EDTA (TE<sup>-4</sup> Buffer), 1X, pH 8.0**

Expiration date: Six months from date of preparation

	Stock	200 mL	500 mL
10 mM Tris	1.0 M	2 mL	5 mL
1mM EDTA	0.5 M	40 µL	100 µL
Type I Water		160 mL	400 mL

Add Tris to Type I Water and adjust the pH 8.0 using 37.1% HCL. Add EDTA, recheck pH and bring to appropriate final volume. Autoclave for 20 minutes and store at room temperature.

For HCL, Tris, and EDTA see this list.

**TRIS-EDTA (TE<sup>-4</sup> Buffer), 100X**

Expiration date: Six months from date of preparation

	MW	1 L	2 L	4 L	8 L
0.1 M EDTA	372.1	37.2 g	74.4 g	148.8 g	297.6 g
1 M Tris	121.1	121.1 g	242.2 g	484.4 g	968.8 g
Type I Water		1L	2L	4L	8L

Dissolve Tris in 800 mL of Type I Water and adjust the pH to 7.5 with 37.1% HCL. Add EDTA, recheck pH and bring solution to final volume. Autoclave for 20 minutes. (It may be necessary to add 10 N NaOH to adjust pH to 7.5.)

For HCL, NaOH, Tris and EDTA see this list.

<p align="center"><b>APPENDIX B: REAGENTS</b></p>	<p align="center">Page 18 of 18</p>
<p align="center"><b>FLUORESCENT DETECTION PCR-BASED STR DNA PROTOCOL:POWERPLEX® 16 BIO SYSTEM - FORENSIC BIOLOGY SECTION PROCEDURE MANUAL, SECTION III</b></p>	<p align="center">Issue No. : 3</p>
	<p align="center">Effective Date: 6-March-2006</p>
<p>UREA</p> <p>Expiration date: Manufacturer's expiration date if listed, otherwise no expiration date</p> <p>Purchased from Sigma, St. Louis, Mo. Catalog Number U-5378, 500 gram bottle.</p> <p>Purchased from Invitrogen, Carlsbad, CA., Catalog Number 15505-050, 2 Kg container.</p> <p>VISUAL MARKER - LAMBDA Hind III (DNA/Hind III)</p> <p>Expiration date: Manufacturer's expiration date</p> <p>Add the appropriate volumes of lambda Hind III and sterile 1X TE-4 Buffer to 250 µL 5X loading buffer (total volume 1250 µL) to achieve a final concentration of 200 ng/10 µL. (The concentration of commercially supplied lambda Hind III may vary from lot to lot.) Mix well and divide into 100 µL aliquots in sterile microcentrifuge tubes. Store at -20 C.</p> <p>NOTE: Lambda Hind III needs to be heated at 65 C for approximately 20 minutes prior to making visual marker.</p> <p>Lambda Hind III - Purchased from Invitrogen, Carlsbad, CA., Catalog number 5612SA, in an approximate concentration of 733 ng/µL, in a total volume of 682 µL.</p> <p>For TE<sup>-4</sup> Buffer and loading buffer see this list.</p> <p align="right"><b>◆END</b></p>	